

RADIATION CARCINOGENESIS AND ACUTE RADIATION MORTALITY IN THE RAT AS PRODUCED BY 2.2 GEV PROTONS<sup>(1)</sup>C. J. Shellabarger<sup>(2)</sup>, R. F. Straub, J. E. Jesseph<sup>(3)</sup>, and J. L. Montour<sup>(4)</sup>Zoology Department, University of Michigan, Ann Arbor  
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The Brookhaven National Laboratory Cosmotron was used to produce 2.2 GeV protons for total-body exposure of young, female Sprague-Dawley rats. A single exposure of 42-day old rats to 92 rads of protons produced a mammary neoplastic response over a 365-day period that was qualitatively and quantitatively similar to the response produced by 158 rads of  $^{60}\text{Co}$  gamma rays. When the two types of radiation exposures were combined they appeared to produce an additive mammary neoplastic response. Because of the qualitative similarity of the mammary neoplastic response to the 2 types of radiation and because the 2 types of radiation appeared to be additive, it was suggested that 2.2 GeV protons act by a mode of action that is similar to that of low LET radiation. Acute radiation mortality produced by a single exposure of 42-day old rats to protons was qualitatively similar in terms of mean survival time to mortality produced by  $^{60}\text{Co}$  gamma rays. It was suggested that 2.2 GeV protons act to produce acute radiation mortality in fashion similar to that of low LET radiation. The administration of a radioprotective drug, AET, to 36-day old rats produced a small decrease in radiation mortality and a small increase in mean survival time when the drug was given before exposure to 2.2 GeV protons. This result was taken to mean that 2.2 GeV protons act at least in part as low LET radiation. Although exact RBE values could not be determined for acute mortality and carcinogenesis in the young rat, approximate values appear to be 1.4 and 1.5.

## INTRODUCTION

When the 2.2 GeV proton facility of the Brookhaven Cosmotron became available for biological studies, proton carcinogenesis, the interaction of protons and gamma-rays on carcinogenesis, proton-induced acute mortality, and chemical protection against proton-induced acute mortality were studied in the rat and these proton-produced responses were compared to similar responses produced by gamma-rays or x-rays.

## MATERIALS AND METHODS

Animals - Weanling, litter-mate, female, Sprague-Dawley rats were obtained from Sprague-Dawley, Inc., Madison, Wisconsin. All rats were kept at the University of Michigan until they were approximately 35 days of age when they were taken to Brookhaven National Laboratory, exposed and returned to the University of Michigan. Litter-mates

were assigned to each experimental and control group so that approximately equal numbers of littermates were placed in each group. Animals to be studied for mammary neoplasia were handled as described previously (ref. 1) for 365 days post-exposure when all animals alive were killed. All animals were examined frequently for mammary tumors and as these were found, they were removed, sectioned and given a pathologic classification. Animals used for acute mortality studies were followed for 30 days and deaths were recorded as to the nearest day post-exposure. Chemical protection studies were done by injecting 2-aminoethylisothiouronium bromide (AET), 30 mg per rat by the intraperitoneal route, 15 minutes before exposure.

## EXPOSURE CONDITIONS

Protons of 2.2 GeV, produced by the Brookhaven Cosmotron were used to expose rats in exactly the

same way as described previously for mice (ref. 2) except that the inside diameter of the lucite tube holding the animals was increased to 4.5 cm and a length of 50 cm. Four rats were exposed per Cosmotron run, nose to tail, with the nose facing the stream of protons. As a check on a possible change in dose with position of the rat within the exposure tube, or change of dose with depth, rat position in the tube was recorded and checked against individual and group rat mortality. The exposure tube holding the rats was placed well within the beam of protons and parallel with the beam as determined by means of a fore and aft gun sight array and Polaroid film. The animals in the exposure tube were rotated along their longitudinal axis at 20 rpm to insure a uniform dose distribution. The Cosmotron beam pulse duration was 1 msec and the repetition rate was 25 pulses per minute. With the proton fluence employed, the instantaneous dose rate was 14 k rads/sec or an average dose rate of 350 rads/min.

Gamma-ray exposures were done at a dose rate of 14 R/min. X-ray exposures were accomplished by operating a conventional x-ray therapy machine at 250 kVp and 30 mA with 0.5 mm Cu and 1 mm Al added filtration at a dose rate of 115 R/min.

#### DOSIMETRY

Each proton irradiation run was monitored by use of the proton activation of the  $^{12}\text{C}$  in a polyethylene foil via the reaction  $^{12}\text{C}(\text{p},\text{np})^{11}\text{C}$  as described previously (ref. 2). The foils were calibrated in terms of dose by means of a tissue equivalent ionization chamber (ref. 3). The foil fluence determination and the dosimetry yielded a result of 354 rads<sup>(1)</sup> per  $10^{10}$  protons/cm<sup>2</sup>.

For x-rays and gamma-rays, the exposure dose was measured in air at the dorsal-ventral midpoint of the animals with a Victoreen ionization chamber in R and these values were converted to rad values, using a physical factor of 0.95 to convert

<sup>(1)</sup>W. H. Moore, Brookhaven calculated, for primary ionization, 332 rads and Wright *et al.* (Health Physics 16, 13, 1969), for total absorbed dose, 410 rads.

R to rads and a biological factor of 0.83 (ref. 4) to relate  $^{60}\text{Co}$  gamma-rays to 250 kVp x-rays.

#### EXPERIMENTS

**Carcinogenesis.** Forty-two day old rats were exposed to 92 rads of 2.2 GeV protons, or 158 rads of  $^{60}\text{Co}$  gamma-rays, or both. The gamma-ray exposure was done approximately 12 hours before the proton exposure.

**Acute mortality.** Forty-two day old rats were exposed to 473, 600, 661 or 729 rads of  $^{60}\text{Co}$  gamma-rays or 354, 478, 595 or 715 rads of 2.2 GeV proton.

**Chemical protection.** Thirty-six day old rats were exposed to 712 rads of 250 kVp x-rays with or without prior AET, or 527, 552, 577 or 602 rads of 2.2 GeV protons with or without prior AET.

#### RESULTS

The mammary neoplastic response to 92 rads of proton exposure, or 158 rads of gamma exposure, or both types of radiation are presented in Table 1 along with mammary neoplastic incidence of non-irradiated litter-mate controls. It seems clear that all measures of mammary neoplasia incidence are approximately the same in response to either 92 rads of proton exposure or 158 rads of  $^{60}\text{Co}$  gamma exposure. Although the value is somewhat uncertain the approximately equal effects of doses of 92 rads from protons and 158 rads from gammas, yield a RBE of about 1.5 using a linear dose-response relationship for each type of radiation and subtraction of control values.

When the proton exposure was combined with the gamma-ray exposure, the percent of rats with mammary neoplasia was not very informative because the dose of each radiation that was selected when combined appears to saturate this measure of mammary neoplastic response. However, the measures of total number of mammary neoplasms, or total number of mammary adenocarcinomas, or the total number of mammary fibroadenomas, when each is corrected for number of rats at risk, appear to indicate that

Table 1

Mammary Neoplasia and Mammary Neoplasms at 300 Days Post-Exposure.  
 Exposures Done at 42 Days of Age.  $^{60}\text{Co}$  Gamma Exposure Preceded  
 Proton Exposure by 12 Hours. AC Refers to Adenocarcinoma and FA  
 Refers to Fibroadenoma.

Treatment	Rats with Mammary Neoplasia			Mammary Neoplasms					
	Rads	N	%	Total	Total N	AC N	AC %	FA N	FA %
None	-	22	1	4	1	0.04	1	0.04	0
Proton	92	32	14	44	20	.62	4	.12	16
$^{60}\text{Co} \gamma$	158	32	16	50	23	.72	4	.12	19
Proton + $^{60}\text{Co} \gamma$	250	28	19	68	37	1.32	8	.29	28
									1.00

proton irradiation and gamma-ray irradiation are very close to being additive. This result implies that the mechanism of action of the 2 types of radiation are similar.

The results of studies on acute mortality in terms of percent dead within 30 days, and the mean survival time are shown in Table 2. Within the lethal range of doses, the mean survival times are about the same for the 2 types of radiation and this implies that proton exposure or gamma-ray exposure produces acute mortality by a similar mechanism. Because the number and the range of doses of each type of radiation is small, and the sample size is not large, a value for RBE cannot be given with any high degree of confidence. However, if these data are plotted, and a visual fit of the survival curves are drawn, and the ratio of  $LD_{50}$  values is determined, the proton exposure rad for rad appears to be about 1.4 times more potent than for the gamma-ray exposure.

Table 2

Acute Radiation Mortality, Number of Animals Exposed at 42 Days of Age, Percent 30-Day Survival and Mean Survival Time in Days of Decedents.

Radiation	Rads	N	% Dead	M. S. T.
$^{60}\text{Co} \gamma$	473	9	0	-
$^{60}\text{Co} \gamma$	600	9	56	16.4
$^{60}\text{Co} \gamma$	661	9	78	17.4
$^{60}\text{Co} \gamma$	729	10	100	10.7
Proton	354	12	33	21.0
Proton	478	12	67	17.5
Proton	595	12	100	9.8
Proton	715	12	100	4.5

The data concerned with the radioprotective action of AET are shown in Table 3. Although all exposure doses used proved to be lethal in the absence of the drug, AET produced a small protective effect against mortality in the 2 lowest doses of proton exposure and the x-ray exposure. A modest increase in mean survival time was produced by AET at all proton exposure doses and the single x-ray dose. It thus appears that AET does have the capacity to protect against acute radiation mortality provoked by 2.2 GeV proton exposure.

Analysis of the position of the rat in the exposure tube by using days of survival time disclosed that survival time increased as the position was moved downstream. This increase was related to reduction in dose - produced increased survival time. This indicates that the decrease in dose received by the last rat downstream is no less than 72% of the first rat and is in agreement with interposed ionization chamber measurements where the dose decreased to 68%.

Table 3

Acute Radiation Mortality, Number of Rats Exposed at 36 Days of Age, Percent 30-Day Survival and Mean Survival Time in Days of Decedents. AET Injected by Subcutaneous Route 15 Minutes Before Exposure.

Radiation	Rads	Drug	N	% Dead	M. S. T.
X-Ray	712		10	100	5.4
X-Ray	712	AET	10	80	9.4
Proton	527		12	100	8.2
Proton	527	AET	12	75	9.7
Proton	552		12	100	7.2
Proton	552	AET	12	92	9.4
Proton	577		12	100	6.1
Proton	577	AET	12	100	9.7
Proton	602		12	100	5.3
Proton	602	AET	12	100	8.2

## DISCUSSION

The results here presented are perhaps of more qualitative interest than of quantitative interest. Although only a single proton dose was tested, it is clear that 2.2 GeV protons have the capacity to accelerate the rat mammary neoplastic response and that this proton-induced mammary neoplastic response is qualitatively similar to the mammary neoplastic response to gamma radiation. This finding confirms a preliminary report (ref. 5) that indicated that the mammary neoplastic response to 2.2 GeV protons and  $^{60}\text{Co}$  gamma-rays was qualitatively similar. Strengthening the conclusion that either proton exposure or gamma-ray exposure produces a qualitatively similar mammary neoplastic response is the finding that when the two types of exposure were combined an additive result ensued. It has been shown previously that x-rays (ref. 6) and protons (ref. 5) produce a linear dose-response. The 12-hour gap between gamma-ray exposure and proton exposure in the experimental group that received both types of exposure probably is of no biological consequence since it has been shown that the incidence of mammary neoplasia of the rat is little changed by short-term fractionation of sub-lethal total body x-ray exposure (ref. 7). It is not unreasonable to suggest, therefore, that the mechanism of action that allows proton exposure to be carcinogenic is not dissimilar to the mechanism of action that allows gamma-ray exposure to be carcinogenic. Since gamma-rays are low LET radiation, then 2.2 GeV protons should be considered to act, in a large part, as low LET radiation.

It seems clear, also, that 2.2 GeV proton exposure produces an acute radiation mortality that is qualitatively similar to that produced by gamma-ray exposure. Again, it is tempting to suggest that 2.2 GeV protons act by a mechanism that is similar to low LET radiation. Adding to this suggestion was the absence of high LET "early death" that has been reported by others who have noted a shift from marrow to gut death with high LET radiations (ref. 8).

It is generally accepted that radioprotective compounds are more effective against low LET radiation than against high LET radiation (ref. 9). Thus, the finding of a small but definite protection with AET against acute mortality as produced

by 2.2 GeV protons may be taken to indicate that these protons act, at least in part, as low LET radiation.

The finding that it is possible to protect against acute radiation mortality as produced by high energy protons by prior AET treatment may be of some interest to those who are concerned with astronauts and their possible exposure to high energy protons. The data here reported are, we believe, the only direct experimental test reported on this subject, and are of some academic interest.

The interrelation between 2.2 GeV proton-produced radiation carcinogenesis and acute mortality is of some radiobiological interest. There is no a priori reason to expect that the relative biological effectiveness of minimum ionizing protons would be the same for tumor induction as it is for inducing mortality because the induction of a tumor must depend upon the number of cells that are capable of division after radiation exposure (ref. 10) while the production of acute radiation mortality depends upon the number of cells of the blood forming organs and the gastrointestinal tract that are not capable of division after radiation exposure (ref. 11). Thus, the finding that the relative biological effectiveness of 2.2 GeV protons was, within the limits of the two experiments, not very different on these two dissimilar biological endpoints was somewhat surprising.

Data published dealing with the biological effects of high energy protons are not extensive. Ueno and Grigoriev (ref. 12) have summarized data dealing with proton energies between 126 MeV and 730 MeV on 52 experiments in mammals including cytological changes, organ atrophy and acute mortality. They believe that a single value of RBE,  $0.82 \pm 0.04$  can be assigned to all of these end points. On the other hand, for 2.2 GeV protons, the results are not so clear. Jesseph *et al.* (ref. 2) reported an RBE value of 0.87 for acute mortality in the mouse. Montour, *et al.* (ref. 13) using mice and spleen-thymus weight loss at 2-3 and 4-5 days after exposure and the same exposure condition and dose measurements of Jesseph *et al.*, obtained RBE values of approximately 1.0. Stoner, *et al.* (ref. 14) using mice and the same exposure conditions of Jesseph, *et al.* obtained a value of 2.5

for the repression of primary tetanus antitoxin responses, 1.5 for secondary responses and 1.5 for enhanced susceptibility to anaphylaxis. Thus, in mice, using similar exposure conditions, RBE values ranging from 0.87 to 2.5 have been reported for different end points. The approximate RBE values obtained in rats of 1.4-1.5 for mortality and carcinogenesis were well within the range reported for mice. This range of RBE values would seem to confirm a previous suggestion of Bond (ref. 15) that the RBE of 2.2 GeV protons may depend upon the criterion of biological effect under study.

Jesseph, et al. (ref. 2), Montour, et al. (ref. 13) and Stoner, et al. (ref. 14) all give reasons for interpreting their data on 2.2 GeV protons as being consistent with what would be expected from low LET radiation. The current data obtained in the rat on carcinogenesis, interaction of protons and gamma-rays on carcinogenesis, acute mortality and chemical protection against acute mortality also are consistent with low LET radiation effects. On the other hand, Jesseph, et al. suggest that as the diameter of the absorbing material is increased, it is possible that a high LET component may become relatively important although biological data on this point are too fragmentary at the present time to allow a conclusion to be reached on this point. All and all, it seems reasonable to think that 2.2 GeV protons act largely as low LET radiation but it must be admitted that the question of a high LET component must remain open.

#### SUMMARY

A single exposure of 42-day-old female Sprague Dawley rats to 92 rads of 2.2 GeV protons produced over 365 days an incidence of mammary neoplasia that was qualitatively and quantitatively similar to the neoplastic response to 158 rads of  $^{60}\text{Co}$  gamma-rays. Both radiations, when combined, appeared to produce an additive neoplastic response. It was suggested that 2.2 GeV protons are approximately 1.5 times more effective than  $^{60}\text{Co}$  gamma-rays. Acute mortality produced by 2.2 GeV protons and  $^{60}\text{Co}$  gamma-rays was qualitatively similar and the proton irradiation appeared to be about 1.4 times more effective than gamma irradiation. A small reduction in acute mortality and a small increase in mean survival time was produced when AET was administered before 2.2 GeV proton irradiation. All of these data were interpreted as indicating that 2.2 GeV proton irradiation produces carcinogenesis and acute mortality in the rat by a mode of action that is similar to that of low LET radiation.

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